March 18, 1880.

THE PRESIDENT in the Chair.

The Presents received were laid on the table, and thanks ordered for them.

The following Papers were read:—

I. "On the Structure of the Immature Ovarian Ovum in the common Fowl and in the Rabbit. To which is appended some Observations upon the Mode of Formation of the *Discus Proligerus* in the Rabbit, and of the Ovarial Glands or 'Egg-tubes' in the Dog." By E. A. SCHÄFER, F.R.S. Received March 3, 1880.

[PLATES 2, 3, 4.]

The Ovarian Ovum of the Bird.

In the ovary of a laying hen, Graafian follicles are to be found in almost every stage of growth, and the fundamental parts—germinal spot, germinal vesicle, yolk, and follicular epithelium—exhibit differences both in relative size and in structure at various stages of growth. I will proceed to consider the structure of each fundamental part in detail, as it is presented in sections of the ovary prepared after hardening in picric acid and alcohol, and stained with logwood or with magenta.

Germinal Spot.—In *larger* ovarian ova, those which are already ·5 millim. and upwards in diameter, the germinal spot appears homogeneous and stains of a uniform dark colour with logwood (fig. 1). It is either completely spherical in shape, or somewhat ovoid. In the latter case it is very commonly eccentric and placed close to the wall of the germinal vesicle, with which it may appear to be blended. When spherical it is often situated near the centre of the vesicle; and from its periphery, in what may be considered a typical condition, filaments of great tenuity radiate outwards to the wall of the vesicle, to the inner surface of which they are again attached, and that so closely that it frequently happens that when shrunken from the action of reagents, the filaments may break in the middle rather than suffer themselves to be dragged away from the wall (fig. 1, *g*). These radiating filaments ramify and intercommunicate in such a way as to form a delicate network (intra-nuclear network) which as we have seen

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serves to connect the germinal spot with the inner wall of the germinal vesicle. The network is hardly at all stained by logwood, thus presenting a marked contrast to the substance of the germinal spot; moreover the nodal points are not marked and the whole structure is comparatively indistinct. It is just such an appearance as can be produced in an albuminous fluid by coagulating reagents, and I am at present unable to decide clearly in my own mind, whether it is natural or has thus been formed artificially. In other ova, chiefly those of smaller size (figs. 2, 3, 4, and 8), the germinal spot is composed of two distinct substances, a homogeneous matrix (*ma.*) staining but slightly, and a number of coarse granules which are darkly stained by the logwood. The granules may be scattered equally thickly over the whole matrix, or they may leave a peripheral layer of the spot clear, or again there may be a clear part in the centre (fig. 3, *c*) as well as at the periphery of the spot(1).* This last condition I have especially noticed in some of the smallest ova. As a rule the intra-nuclear network above described in the larger ova is not to be seen in the smallest ones. This may be accounted for by the assumption that the network is formed subsequently by a protrusion of the homogeneous substance of the spot; *i.e.*, if we regard the network as a natural formation. On the other hypothesis, namely, that it is an artificial production, we must assume that there is less albuminous matter in the fluid which fills the germinal vesicle of the smaller ovum, to what there is precipitated around the spot, and does not adhere together in the form of reticulating filaments. In almost all these smaller ova the germinal spot lies near the wall of the vesicle.

Germinal Vesicle.—In all the ova the wall of the germinal vesicle is a very distinct membrane which becomes stained by logwood, but not very darkly. When a portion of the membrane of the germinal vesicle of one of the larger ova is separated and examined on the flat, it has a finely punctated appearance, and in profile it can be seen that this is due to minute pits with intervening thickenings upon the inner surface of the membrane (fig. 7, *a* and *b*). (2) The action of the reagents employed generally produced a shrinking of the vesicle, so that its outline in many cases became irregular (fig. 3), but where this shrinking has not taken place, it is almost perfectly spherical except in the largest ova. With the exception of the germinal spot the contents of the germinal vesicle in the younger ova remain absolutely clear and unstained, so that we must either assume that there is very little albuminous matter naturally present, or, as above suggested, that this has been precipitated around the germinal spot during the process of hardening the tissue. In one small ovum two

* The small numbers above the line indicate references to the literature of the subject. These will be found at the end of the paper.

germinal vesicles were observed in close contiguity. They were of unequal size, and each had a relatively large macula. In another, a figure of which is given (fig. 8), there are also two vesicles, but one of these is evidently in process of disappearance, for the membrane has become absorbed and the contents of the vesicle, including the macula, are becoming assimilated in appearance to the vitellus.

Yolk.—In all the ova the yolk seems composed of a network of closely interlacing filaments (3). The network may be coarser or finer; it is very fine indeed in the peripheral layers of the largest ova (those above a millimetre in diameter) which generally have a finely granular aspect (fig. 10, I, II); coarser in the smaller ova and in the central parts of the larger, but in the latter the coarse appearance of the network seems chiefly due to the accumulation here of the large spherical yolk globules, which being left clear in the sections, give a reticulated appearance to the substance in which they are imbedded. In the smallest ova there is nothing but clear fluid in the meshes of the network, which is comparatively open. But in almost all there are condensations of the network (fig.6, *c*,) to be seen, in which the filaments are far more closely interlaced, the appearance presented being very like that which might be produced by gathering up a net made of string into a bunch at one part. The filaments of the vitelline substance are but slightly stained with logwood, but since they are more closely placed in the condensations here spoken of, these are more stained than the rest of the yolk. Eventually as the ova increase in size the condensations seem to become more numerous (fig. 5), and to spread, and the vitelline network to become eventually closer and finer everywhere, but especially at the periphery of the ovum and around the germinal vesicle, the deposit of the yolk globules producing, as we have seen, a coarser appearance in the more central parts.

Other condensations (fig. 9) of vitelline substance occur in some ova which are more circumscribed and definite than those just mentioned. They are nucleus-like, spheroidal patches, which stain deeply with magenta, less deeply with logwood, are homogeneous in appearance, and are usually encircled with a corona of short, straight, radiating filaments, which do not interlace with one another like those of the vitelline substance around, but may however (I am not sure of this) be continued into the vitelline network of these *pseudo-nuclei*, as I will term the structures in question. There may be one, two, or even a greater number in the vitellus of the same ovum, with the germinal vesicle of which they have no apparent connexion.

I am unable to conjecture the meaning of these pseudo-nuclei, or to decide whether they are of the same nature as the "yolk-nuclei" which have been mentioned by several observers as occurring in the ova of fish and Amphibia (4).

In the largest ova the yolk exhibits several distinct layers which

shade off the one into the other. Close to the surface is a thin layer (fig. 10, I) very clear and almost free from granules, or these are of extreme minuteness; next there is a finely granular looking layer (II), considerably thicker than the last, in the deeper part of which are imbedded the elements of the white yolk (III), which thus forms the third layer, and which passes gradually into the main central substance, or yellow yolk (IV). It is difficult to observe the interlacing filaments of which the vitelline substance is in all probability composed: even with the highest powers the superficial layers of the yolk of these larger ova show little more than an appearance of fine punctation.

Peculiar striated systems of fibres (figs. 12 and 13) are to be seen occasionally in the vitellus of some of the larger ova. They are generally situated near the end or ends (for there may be two such systems in one ovum) of the ovum, and call to mind some of the amphiastral appearances of dividing nuclei. The fibres composing them do not become more darkly stained with magenta than the rest of the vitellus, but they are very obvious both on account of their peculiar arrangement (each system consisting of two triangular halves diverging from a common centre) and on account of the fact that they lie in a substance which is clearer and stains less than the rest of the vitellus.

In the ovum figured (fig. 12) one of these striated systems is situated in a projection of the vitellus. I draw attention to this because it may mean that the vitellus just at the neck of the projection where the system is situated is in a condition of contraction, and that the appearance described is characteristic of that condition. On the other hand, it is just possible that the figures in question are actually karyolitic, and derived from some extruded portion of the contents of the germinal vesicle. In this case the phenomenon is probably connected with the precocious formation of polar globules. I say "precocious," because the ovum in question was far from maturity ([5]).

Membranes of the Ovum.—I have only one remark to make about the external coverings of the bird's ovarian ovum, and it concerns the *zona radiata*. Waldeyer states that this membrane or layer is derived from cells of the follicular epithelium, and is continuous with their substance ([6]). I find on the contrary that in good sections the zona is completely distinct from the follicular epithelium, and its outline is more sharply defined externally than internally (fig. 11, *z. r.*). Moreover, where the epithelium has accidentally been broken away, portions of its cells do not adhere to the zona, as they would tend to do if their substance were continuous with it. I am inclined with Balfour ([7]) to regard the *zona radiata* therefore as a product of the protoplasm of the ovum. I have not been able to gain any information with regard to the origin of the vitelline membrane which might enable me to decide whether it is a product of the yolk or of the follicular epithelium.

Nor can I say whether the clear interval between the follicular epithelium and the *zona radiata* in fig. 11, and which seems to be bridged across here and there by processes of the follicular epithelium cells, is the section of a homogeneous "chorion," or merely a layer of clear coagulated fluid (8).

Epithelium of the Graafian Follicle.—The follicular epithelium of the smallest follicles is a single layer of oblong or cubical cells (figs. 5, 6, 8, 9, *f. e*). As the follicle grows the cells become more columnar. In larger follicles (figs. 10 and 11) the epithelium is two or more cells deep (9). The cells are not arranged in layers, but irregularly disposed. They are for the most part rounded or angular in form, but between these smaller cells other longer ones are met with, which extend through the thickness of the epithelium, and are pyriform in shape, generally with the stalk of the pear directed outwards (fig. 10, *p*). These seem to be the cells which Waldeyer figures as being continuous with the *zona radiata*, but, as will be seen from what has been stated above, I have not been able to confirm their continuity; indeed, the larger end of the cell does not always reach the inner border of the follicular epithelium. On the other hand, the stalk of the pear always extends at least to the outer border of the epithelium, and may even in some cases be traced into the connective tissue coat of the follicle. These cells call to mind the pyriform cells which cover the surface of the developing Elasmobranch ovary, and which also are traceable at their attached smaller ends into the stroma of the organ (10).

Ovarian Ovum of the Rabbit.

The following description is derived from the study of sections of the ovaries of rabbits, which were either full grown or nearly so. The ovaries were removed while still warm and placed in a concentrated solution of picric acid. After hardening in this, they were transferred to alcohol, which was frequently changed. Sections were then prepared and stained either with magenta dissolved in oil of cloves, and subsequently mounted in Canada balsam, or they were stained with Beale's carmine and mounted in glycerine, or sometimes in balsam. In sections of such ovaries, as is well known, permanent ova and Graafian follicles are to be found in every stage of growth.

Germinal Spot.—In small ova the germinal spot, as in the bird, is generally represented by a collection of darkly staining granules, imbedded in a homogeneous, or very finely granular matrix (figs. 14—20). The outline of the macula is generally less well defined than in corresponding ova of the bird, and the shape is more irregular. This granular condition of the macula is not confined to the small ova, for it occurs also in those which are surrounded by a double layer of follicular epithelium (fig. 20), and even in some which are contained in large Graafian follicles in which there is already a considerable

accumulation of *liquor folliculi*. In the latter case the matrix above-mentioned is absent, and the macula is represented by a number (a dozen or so) of globules of varying size which appear to lie loose within the germinal vesicle (fig. 23, *b*). An intra-vesicular network is sometimes present, and serves to unite the granules of the macula, which are then few in number and individually larger (figs. 21 and 22). It is possible that the homogeneous matrix above described may represent the remains of such a network, the filaments of which have shrunk up into a mass on contact with the hardening reagent; but if we are to assume this for some cases, it is difficult to understand why it should not take place in every case.

Germinal Vesicle.—With the exception of the germinal spot or spots, and the intra-vesicular network when present, the contents of the germinal vesicle are perfectly clear and do not become stained to any appreciable extent. The vesicle is either completely spherical in shape or it may present one or more bulgings (fig. 18), and these bulgings are frequently turned towards the nuclei presently to be described as making their appearance in the peripheral layer of the vitellus. In some of the sections the wall of the germinal vesicle seems to be deficient for a small extent (fig. 14, *v. g.*), as if to permit of an admixture of its contents with the yolk; but this appearance may be due to the wall having become so thinned as to be imperceptible. In some of the smallest Graafian follicles two germinal vesicles are seen within one ovum (fig. 26). It is possible that one of these may afterwards disappear, *i.e.*, that the follicle is really a nest, containing two fused primitive ova; or, on the other hand, that the follicle and ovum are about to divide.

Vitellus.—In small ovarian ova the vitellus, as in the corresponding ova of the bird, is in the form of a comparatively open network of anastomosing filaments (figs. 21, 22). These tend to become collected more thickly together immediately around the germinal vesicle, and also at the periphery of the vitellus. In larger ova a network is no longer recognisable, but the vitellus acquires a uniformly granular aspect, with generally a number of larger and more distinct granules imbedded in it. In maturing ova the vitelline granules are chiefly collected immediately in contact with the inner surface of the *zona pellucida*, but are also distributed throughout the whole vitellus, disposed, it has sometimes appeared to me, in closely anastomosing tracts, leaving clear intervals between.

The *zona pellucida* of the rabbit's ovum is late in becoming formed. Even when the epithelium in the Graafian follicle is composed of two layers of well-marked columnar cells, it is still thin and the radiating pores cannot be detected in it. In later stages of growth the pores are distinct enough, and they often appear to be occupied by minute granules which are like the vitelline granules on the one hand, and, on

the other hand, are indistinguishable from those in the layer of granular material which lies between the outer surface of the *zona pellucida* and the innermost layer of cells of the *discus proligerus* (fig. 23, *a*). There can be little doubt that this granular matter is produced by the cells of the discus, and I cannot but conjecture with Waldeyer ([11]) that the granules in question pass bodily through the pores of the zona to add themselves to the food material of the vitellus. I have seen no appearances such as those represented by Pflüger ([12]) and Lindgren ([13]) of the passage of entire cells of the discus through the zona ([14]).

Follicular Epitheluim.—In the smallest Graafian follicles which are met with in the ovary of the grown rabbit, the epithelium of the follicle consists of a single layer of flattened cells, which tend, however, to be thicker and more cubical on one side of the follicle, that, namely, which is further from the surface of the ovary (fig. 25) ([15]). As the ovum and follicle grow, the epithelium cells multiply and become uniformly columnar, but long remain in a single layer (figs. 14, 15, 16, 18, 21). Presently another layer is formed within this first one (fig. 17). This inner layer of the follicular epithelium (*discus proligerus*) in the rabbit appears to be formed at the expense of the ovum. At least, this is the only interpretation which I can adopt of the remarkable appearances I am about to describe.

Formation of the Discus Proligerus in the Rabbit.—If we examine those Graafian follicles in the rabbit's ovary whose follicular epithelium is still composed of but a single layer of well-marked columnar cells, it may frequently be observed that one or more nuclei are imbedded in the peripheral layer of the vitellus (fig. 14, *n*), and this part of the vitellus may be marked off from the rest by a sector (fig. 16). The nuclei in question are oval, with their long diameter parallel to the circumference of the ovum, and at right angles therefore to the long axis of the columnar cells and oval nuclei of the follicular epithelium. The protoplasm in which they lie is more sharply marked off from this epithelium than it is from the vitellus, and, indeed, it looks like a part of the latter cut off, for it has the same finely granular appearance, is stained similarly to that with carmine and magenta, and is bounded externally by a direct continuation of the general circumferential line of the ovum. Some ova are met with which have only one of these nuclei, others, in which there are two or more. When more than one, they may make their appearance in different parts of the periphery, and these parts being segmented off, the ovum, from being round may acquire a very irregular shape (fig. 22).

As before mentioned, the germinal vesicle is often bulged towards that part of the periphery of the vitellus where the nuclei are situated (fig. 18), and with care fine striations may occasionally be detected traversing the interval. Such appearances incline one to the

belief that the nuclei are derived from the vesicle, but scarcely by a distinct process of cell-division. At least, I have never been able to detect anything in the shape of a division-spindle. The only other possible explanation is that the nuclei and cells in question are derived from cells of the follicular epithelium, which are situated in a different plane from that included in the section, and burrow obliquely between the first formed layer and the vitellus of the ovum, imbedding themselves in the surface of the latter. But this would not account for the sharpness with which they are always marked off from the follicular epithelium, and the fact that both nuclei and cell-substance differ materially in appearance and in their reaction to staining fluids from the nuclei and cell-substance of the first formed layer of epithelium.

However first formed, they soon multiply and spread around the whole of the ovum, so as to form a second complete layer of follicular epithelium, the cells of which in parts may be two deep. They maintain for a time their disposition parallel to the circumference of the ovum and transverse to the direction of the cells of the first formed layer (fig. 17). Moreover, they still exhibit the same behaviour towards staining fluids as the vitellus itself. But as the follicle continues to grow, and as the *zona pellucida* becomes formed between these cells and the vitellus, they acquire a columnar form with the long axis of the cell perpendicular to the surface of the ovum, their contents clear up, and they come to resemble the cells of the first formed layer in every particular (fig. 20).

The only statement that I know of which suggests a parallel between these appearances in the rabbit's ovum and others of a similar nature elsewhere occurs in the description which Kuppfer gives of the formation of an inner layer of follicular epithelium (which is said to develop into the test) from nuclei which make their appearance in the peripheral layer of the vitellus of the ovum of *Ascidia canina* ([16]). Unless it is permitted to cite the observations of Kleinenberg upon the formation of a layer of cells from the periphery of the ovum of *Hydra*, which cells take no part in the development of the ovum, but are simply transformed into a capsular envelope ([17]).*

I have not been able to see anything of the kind in the Graafian follicles of other Mammals (cat, dog, man).

Ovarian Ova of the Bitch.

The young ova (figs. 27 to 29) have a germinal vesicle which contains a germinal spot and a beautiful intravesicular network. Attached to one side of the germinal vesicle is the main part of the solid substance of the vitellus, and this vitelline mass is connected

* There is one important difference between these cases and the appearances in the rabbit's ovum above described, viz., that in them the separation of cells from the vitellus occurs after fertilisation.

to the periphery of the ovum by branching processes. The rest of the contents of the ovum is clear.

Post-Embryonic Formation of Graafian Follicles in the Bitch.

In sections of the ovary of a bitch-pup of eighteen days it is easy to convince oneself of the glandular mode of formation of the Graafian follicles and their contents. Figs. 30 to 33 are representations of such ovarial glands, showing their communication at this period with the surface of the ovary. In fig. 30 this communication is direct, and the whole structure bears a striking resemblance to a racemose gland whose saccules are filled with cells; in fig. 31 the glands are longer and more irregular, and the duct, if we may call it so, does not pass straight to the surface, but in an oblique or even convoluted manner. It is impossible to confound glandular tubes of this description with creases upon the surface of the ovary, as Foulis suggests that Pflüger and Waldeyer have done ([18]), especially since in many cases where the tubes are cut across, the lumen can be distinctly seen (Fig. 32).

We can see in the figures how portions of these solid columns of cells are becoming cut off into "egg-nests" by ingrowths of the ovarial matrix. In many of these nests the primitive ova are already distinguishable from the other cells by their size and the clearness of their protoplasm. But it often happens that the distinction does not appear until the group of cells which constitutes the nest is completely separated from the rest of the egg-tube. Fig. 24 from the rabbit's ovary illustrates the same fact.

In sections of the ovary of a young but nearly full-grown bitch the same glandular character of the superficial parts of the egg-tubes of Pflüger is evident (fig. 34). ([19]) In most the open communication with the surface is lost, and the solid ends of the glands, which form the "egg-columns," properly so-called, have also become severed, and have sunk into the ovarial stroma. But the lumen of the tube is still distinct, and the section is wonderfully like that of the duct of a small secreting gland.

Appearances like these seem to me to point very clearly to the conclusion that the typical mode of formation of the Graafian follicles and their contents is by a gland-like ingrowth of the ovarial epithelium, and that the process, which is described by Balfour, as occurring in the Elasmobranch and in the rabbit, is a modification of this typical mode. For it must certainly be admitted that the deeper-seatedness of the columns of germinal epithelium as the ovary advances in development, cannot be wholly due to the outward growth of the ovarian stroma, since we see how that even after the ovary has attained its full size the Graafian follicles (which are derived from

these columns), as they successively mature, sink deeper into the substance of the organ.

It will be seen that the view which I have been led to take of the derivation of the ova and epithelium cells of the Graafian follicles, is that both structures originate in common from the germinal epithelium—in the dog (Pflüger, Waldeyer) in the form of ingrowths which may closely simulate the formation of tubular glands (ovarial glands); in the rabbit (Balfour) in the form of columns of cells which are altogether solid, and which thus present a modification of what is in all probability, as H. Ludwig's extensive comparative researches have shown, the typical mode. This is essentially the view first enunciated by Pflüger ([20]) for the vertebrate ovary, and differs materially from that of Foulis, who contends for the derivation of the follicular epithelium from the connective tissue cells of the ovarian stroma. I think that Dr. Foulis could not possibly have arrived at this result had he at first selected the ovary of the dog for study. Above all the investigation of the human ovary in this respect teems with difficulties, both because it cannot be obtained fresh enough, and on account of the great relative development of the stroma. On Dr. Foulis' hypothesis that the first formed layer of follicular epithelium is only modified connective tissue, the development in the rabbit of the inner follicular epithelium from the ovum itself, as I have described it, would be quite incomprehensible, whereas, if both ova and epithelium have a common origin, there would be no morphological absurdity involved in this apparently exceptional mode of development; especially since, as V. Beneden ([21]) and Balfour have shown, the permanent ova are for the most part produced by the fusion of two or more cells (primitive ova), which originate from the germinal epithelium in common with the first formed layer.

REFERENCES TO LITERATURE.

1. Compare Eimer ("Untersuchungen ueber die Eier der Reptilien," I. "Archiv für Mikroskopische Anatomie," VIII, p. 218.)
2. Eimer (*loc. cit.*, p. 119) describes actual pores as existing in the much thickened membrane of the germinal vesicle of the ova of some reptiles.
3. This appearance has been remarked by several observers in hardened ova of the fish, reptile, and bird, but evidence is wanting to prove that it may not be a precipitation effect of the hardening reagent.
4. The pseudo-nuclei here described are very different in appearance from the yolk-nucleus which Dr. Allen Thomson has figured in the ovum of the frog ("Cyclopædia of Anatomy and Physiology," Article "Ovum," fig. 65B); nor do they in any way resemble the "Dotterkerne" and "Dotterschorfe," which Eimer describes in reptilian (*loc. cit.*, pp. 225 and 226) and Balfour in Elasmobranch ova ("Structure and Development of the Vertebrate Ovary," "Quarterly Journal of Microscopical Science," vol. xviii, p. 411).
5. The systems of fibres here described may be analogous to the pyramidal systems

of striæ described by A. Schultz, in ovarian ova of the Torpedo. ("Zur Entwickelungsgeschichte des Selachier-eies," "Arch. f. mikr. Anat.," XI, p. 577.)

6. Waldeyer, "Eierstock und Ei," p. 62, and fig. 25.
7. Balfour, *loc. cit.*
8. Gegenbaur ("Eier der Wirbelthiere mit partieller Dotterfurchung, Müller's Archiv." 1861, p. 517) has described a similar homogeneous layer in the reptilian ovum.
9. This is certainly the case in spite of Waldeyer's reiterated assertion to the contrary (*loc. cit.*, p. 58; and article "Eierstock," in Stricker's "Handbuch der Gewebelehre," p. 557).
10. Balfour, *loc. cit.*, p. 391.
11. Waldeyer, "Eierstock und Ei," pp. 40 and 47.
12. E. F. W. Pflüger, "Untersuchungen zur Anatomie und Physiologie der Eierstocke der Säugethiere." 4te. Mittheilung. "Med. Centralzeitung," 1862. No. 90.
13. Hj. Lindgren. "Ueber das Vorhandensein von wirklichen Poren-kanälchen in der *zona pellucida* des Säugethieres," &c. "Arch. f. Anat. u. Phys." Anatomische, Abtheilung, 1877, p. 360.
14. Wagener (Bemerkungen ueber den Eierstock und den gelben Körper," "Arch. f. Anat. u. Phys." Anatomische, Abth. 1879, p. 181) describes and figures a connexion of the cells of the *discus proligerus* with the substance of the vitellus through the pores in the *zona pellucida*.
15. Compare Balfour, *loc. cit.*, p. 427.
16. Kuppfer. "Die Stammverwandtschaft zwischen Ascidien u. Wirbelthieren," "Arch. f. mikr. Anat.," V, 459.
17. N. Kleinenberg. Hydra. Leipzig, 1872.
18. J. Foulis, "On the Development of the Ova and structure of the Ovary in Man and other Mammalia." "Transactions of the Royal Society of Edinburgh," 1875, p. 362.
19. Compare Waldeyer, *loc. cit.*, pp. 30 *et seq.*
20. "Untersuchungen," &c., 3te Mittheilung. "Med. Centralzeitung," 1862, No. 88; and in his important monograph, "Ueber die Eierstocke der Säugethiere und Menschen," 1862.

    F. Valentin ("Ueber die Entwickelung der Follikel in dem Eierstocke der Säugethiere," "Müller's Archiv.," 1838) described the glandular tubes in the ovary, but failed to recognise their connexion with the ovarial epithelium—the true nature of which was not indeed recognised until Waldeyer drew especial attention to it.
21. Ed. van Beneden. "Composition et signification de l'œuf," 1868.

DESCRIPTION OF THE FIGURES.

Figs. 1 to 13 are from sections of hens' ovaries, portions of which were hardened first in picric acid, and subsequently in alcohol. The sections were made after imbedding by the method of soaking with cacao-butter ("Course of Practical Histology," p. 92), and were stained either with logwood or with magenta.

Fig. 1. Germinal vesicle, germinal spot, and intra-vesicular network of a small ovarian ovum (·305 millim. in diameter). Magnified 250 diameters.

m. v. Membrane of germinal vesicle.

g. Break in the intra-vesicular network.

Fig. 2. Germinal spot of small ovum showing matrix and imbedded granules composing the macula.

m. v. Membrane of germinal vesicle to which the macula is adherent.

ma. Homogeneous matrix of germinal spot in which darkly stained granules are imbedded.

Diameter of ovum ·488 millim. Magnified 250 diameters.

Fig. 3. Germinal vesicle and germinal spot of a very small ovum.

m. v., *ma.*, as before.

c. Central clear part of macula. (Diameter of ovum ·122 millim.; of germinal vesicle ·054 millim.) Magnified about 600 diameters.

Fig. 4. *Macula germinativa* of a very small ovum. (Diameter of ovum ·122 millim.; of macula ·049 millim.) Magnified 400 diameters.

Fig. 5. Small Graafian follicle with ovum, showing two or three nucleus-like condensations of the vitellus. (Diameter of ovum ·3 millim.) Magnified 120 diameters.

Fig. 6. Smaller ovum (·264 millim.) than that represented in fig. 5, but more highly magnified, showing a condensation, *c*, of the vitelline network.

Fig. 7. Small piece of the wall of the germinal vesicle of a moderately sized ovum (1·07 millim., germinal vesicle ·195 millim).

(*a.*) On the flat.

(*b.*) Seen in optical section.

The membrane is seen to have a granular appearance.

Fig. 8. Very small ovarian ovum (diameter ·11 millim.) with follicular epithelium, germinal vesicle, and germinal spot; and with a peculiar insulated body in the vitellus, which is apparently the remains of a second germinal vesicle and spot in process of disappearance.

Fig. 9. Part of a small ovum (·3 millim.) with two pseudo-nuclei in vitellus.

f. e. Follicular epithelium.

y. Yolk substance forming a network.

g. v. Part of the germinal vesicle.

Fig. 10. Superficial layers of a comparatively large ovum (1·586 millim.)

f. e. Follicular epithelium.

p. Pyriform cell.

I. Clear superficial layer of vitellus; II. Finely granular layer—its granules are largest near tho first layer; III. White yolk; IV. Superficial part of yellow yolk. Drawn under a magnifying power of about 500 diameters.

Fig. 11. Superficial part of a still larger ovum (2·44 millim.).

f. e. Follicular epithelium.

z. r. Zona radiata.

Fig. 12. Thick section of a comparatively large ovum, showing the peculiar systems of fibres described in the text. Slightly magnified (about 10 diameters).

Fig. 13. The upper part of the previous figure. Magnified 40 diameters.

Figs. 14 to 26 are from sections of rabbits' ovaries.

Fig. 14. Small Graafian follicle, the follicular epithelium forming a single layer of columnar cells.

v. g. Germinal vesicle, the wall of which seems to be deficient at this part.

m. Macula, composed of a mass of granules, imbedded in a homogeneous matrix.

n. Nucleus in the periphery of the vitellus. 570 diameters.

Fig. 15. Small Graafian follicle, not yet completely cut off from the rest of the

"Ei-schlauch." The germinal vesicle seems to be connected by fine lines with two small nuclei at the periphery of the vitellus.

Fig. 16. Small Graafian follicle, similar to that represented in Fig. 14. At one side two nuclei are seen in the peripheral part of the vitellus. The area in which they lie is marked off by a line from the rest of the vitellus. Indistinct striæ pass from here towards the germinal vesicle. 570 diameters.

Fig. 17. Graafian follicle, with a complete inner layer of follicular epithelium. This differs markedly from the outer layer in the appearance and arrangement of the cells. The irregular outline of the ovum is to be remarked. Drawn under a magnifying power of about 350 diameters.

Fig. 18. Small follicle, with single layer of epithelium. Germinal vesicle presents a bulging on that side of it which is turned towards a nucleus-like body in the periphery of the vitellus. The figure is otherwise like figs. 14 and 16, but somewhat less magnified.

Fig. 19. Small follicle, showing three cells in periphery of vitellus. Magnified about 240 diameters.

Fig. 20. A Graafian follicle, much further advanced towards maturity than any of those above represented. The cells of the inner layer of the follicular epithelium have by this time assumed their characteristic columnar form and radiating arrangement. The *macula germinativa* is, as before mentioned, formed of a homogeneous matrix, with dark granules imbedded in it. The *zona pellucida* is now distinct. Magnified 570 diameters.

Fig. 21. Small follicle, with ovum. An intra-nuclear network connects a small germinal spot with the wall of the vesicle. The vitellus is in the form of a network of filaments. Magnified about 200 diameters.

Fig. 22. Small follicle, with outer layer of epithelium complete and a few cells of inner layer formed between this and the ovum. The germinal vesicle contains three small germinal spots united by a network. The vitellus also exhibits a network of delicate filaments. Magnified about 450 diameters.

Fig. 23 *a*. A small portion of the *zona pellucida* of an ovum from a large Graafian follicle, together with some of the cells of the *discus proligerus*, which are mixed with it. A layer of granular substance is seen to intervene between these cells and the outer surface of the zona, and granules similar to those of this substance can be traced through pores in the zona into continuity with the granules of the vitellus. Drawn under a power of about 750 diameters.

Fig. 23 *b*. Germinal vesicle of an ovum from a large follicle.

m. v. Membrane of vesicle. The *maculata germinativa* is in the form of a number of spherical globules, one of which is much larger than the rest. The remainder of the contents of the vesicle is clear.

Fig. 24. A row of four so-called "nests" from near the surface of the ovary of a young rabbit. The egg-cell has not yet increased in size beyond the other cells of the nest. Magnified about 450 diameters.

Figs. 25 and 26. Two very small follicles, with young ova. In fig. 26 two germinal vesicles are seen. From the same ovary and magnified to the same extent as fig. 24.

Figs. 27 to 35 are from sections of the bitch's ovary.

Figs. 27, 28, and 29 represent small Graafian follicles, containing young ova. In each the germinal vesicle is seen to contain an intra-vesicular network, and the vitellus is collected into a patch which is in contact

with the germinal vesicle, and is connected with the periphery of the ovum by ramified offsets.

Fig. 30. Section of superficial part of ovary of a bitch pup, eighteen days old, showing gland-like connexion of egg-tubes with the surface of the ovary.

str. Ovarial stroma.

Fig. 31. From another section of the same ovary, showing more oblique connexion of egg-tubes with the surface.

o. e. Ovarial epithelium. Stroma not represented.

Fig. 32. From another part, showing two other of the gland-like connexions cut across and obliquely.

Fig. 33. A very oblique connexion, cut longitudinally.

Fig. 34. Section of superficial part of ovary of a young bitch, almost full-grown, showing some of the ovarial glands (connexions of egg-tubes with surface of ovary). Cut across and obliquely.

Fig. 35. A peculiar convoluted tube from the deeper part of the same ovary, almost filled with long, tapering columnar cells.

a. Either the same or a similar tube, cut across.

II. "Researches into the Colouring Matters of Human Urine, with an Account of the Separation of Urobilin." By C. A. MacMunn, B.A., M.D. Communicated by A. Gamgee, M.D., F.R.S., Brackenbury Professor of Practical Physiology and Histology in Owens College, Manchester. Received March 6, 1880.

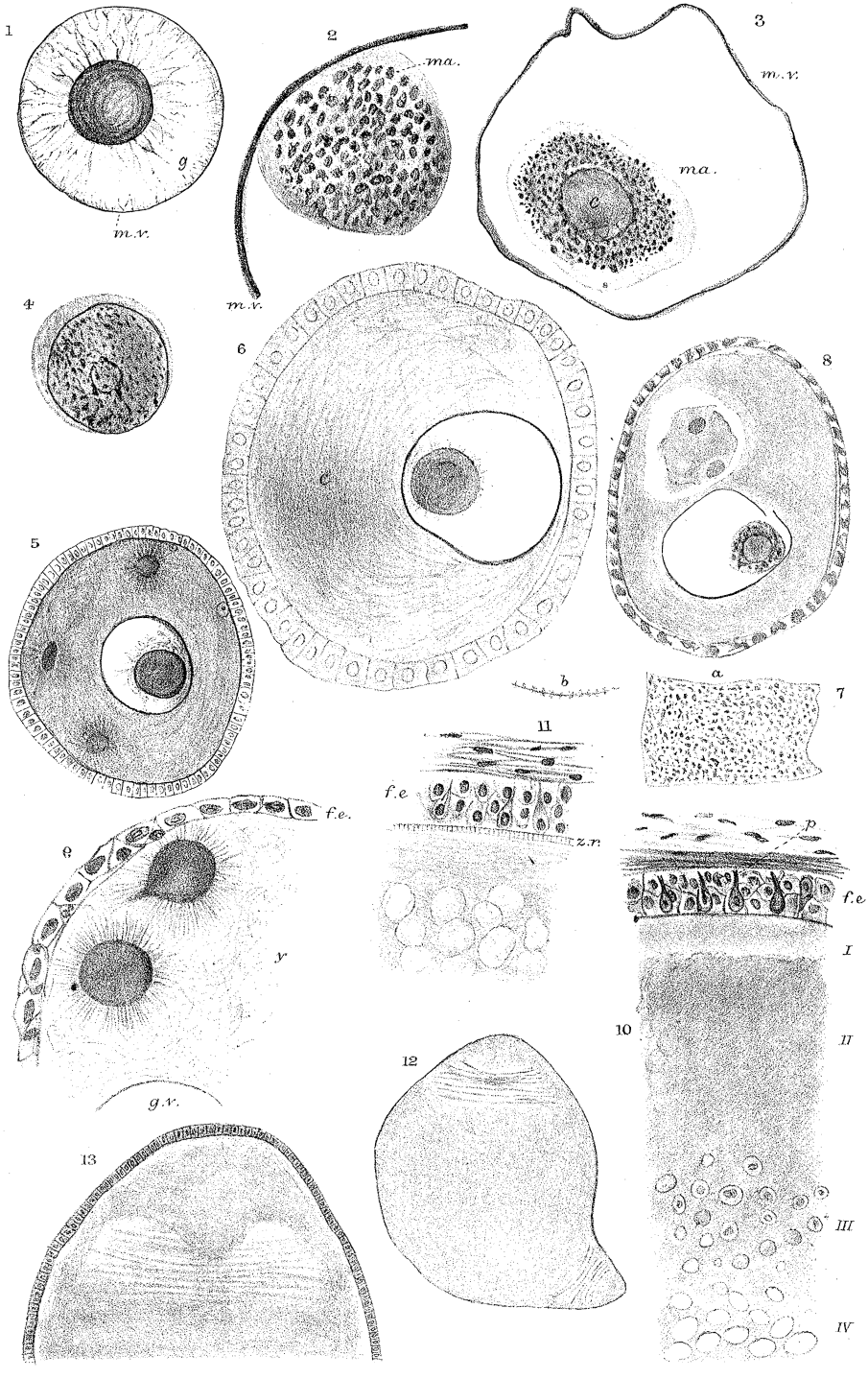
(Abstract.)

In this paper an account of the spectroscopic and chemical characters of urobilin is given. Urobilin is a pigment which has been diagnosed in urine by means of the spectroscope, and which has not been hitherto isolated.

Before its discovery in urine a pigment had been obtained by Jaffé by acting on human bile with nitric acid, and on dog's bile with hydrochloric acid, and subsequent treatment, which in solution gave the same spectrum, and behaved in the same manner with reagents as urine containing urobilin. By an examination of the bile of seventeen animals, I have shown that it is present as such in the bile of all them, but in greatest abundance in the bile of the mouse; and the results are described in the paper, while the spectra are shown in Chart II.

I have also shown that human urine always gives an absorption band at F, which almost always is affected in the same manner by reagents as the pigment got by Jaffé from bile, and that if this band is not so affected another pigment called urolutein is present, which I describe and have mapped in Chart I.

I have succeeded in isolating urobilin by the following method:—Having procured some urine which by preliminary spectroscopic and



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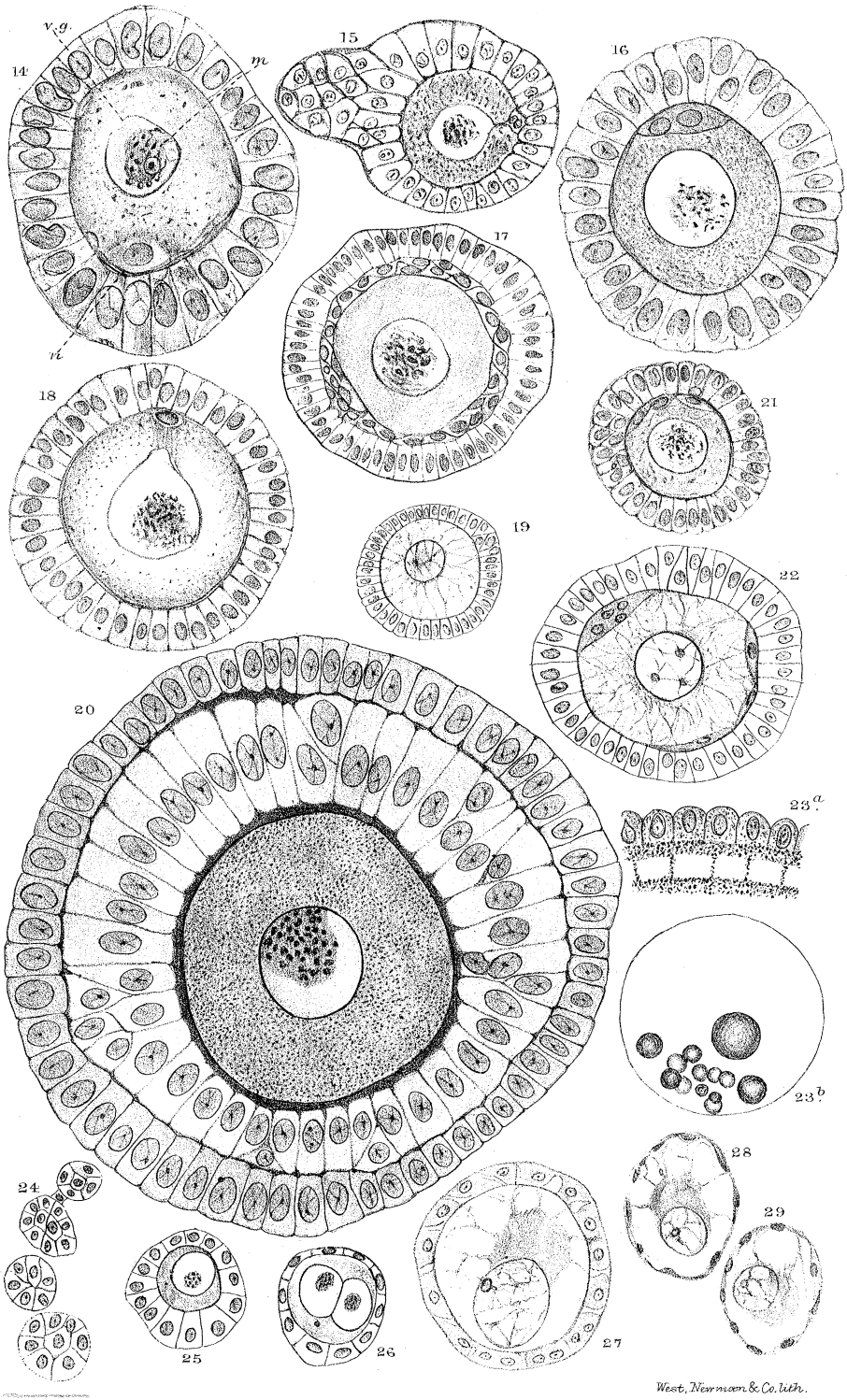

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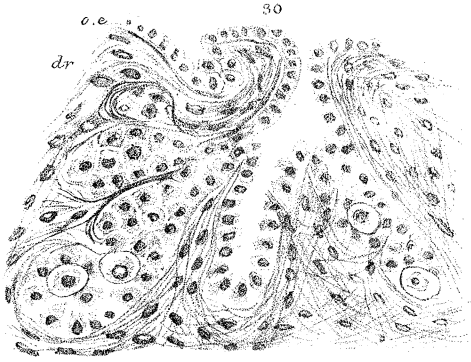

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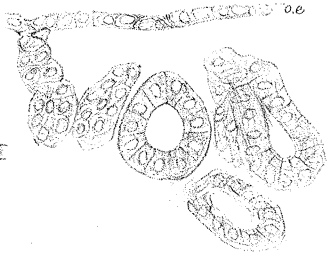

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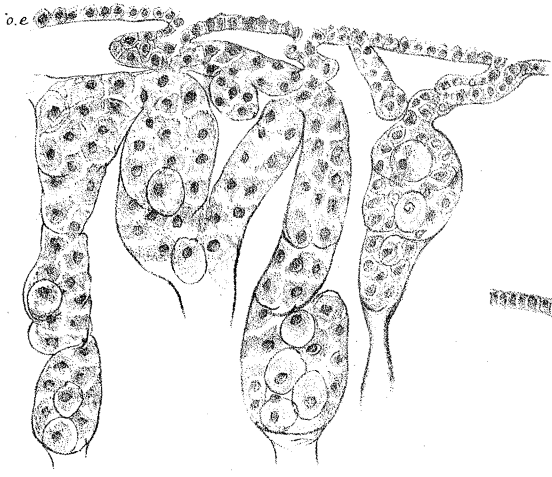

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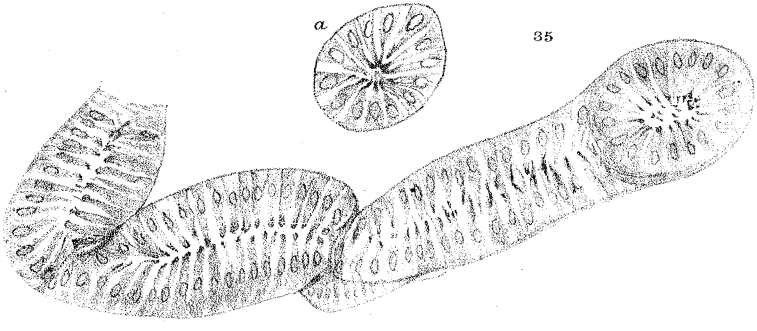

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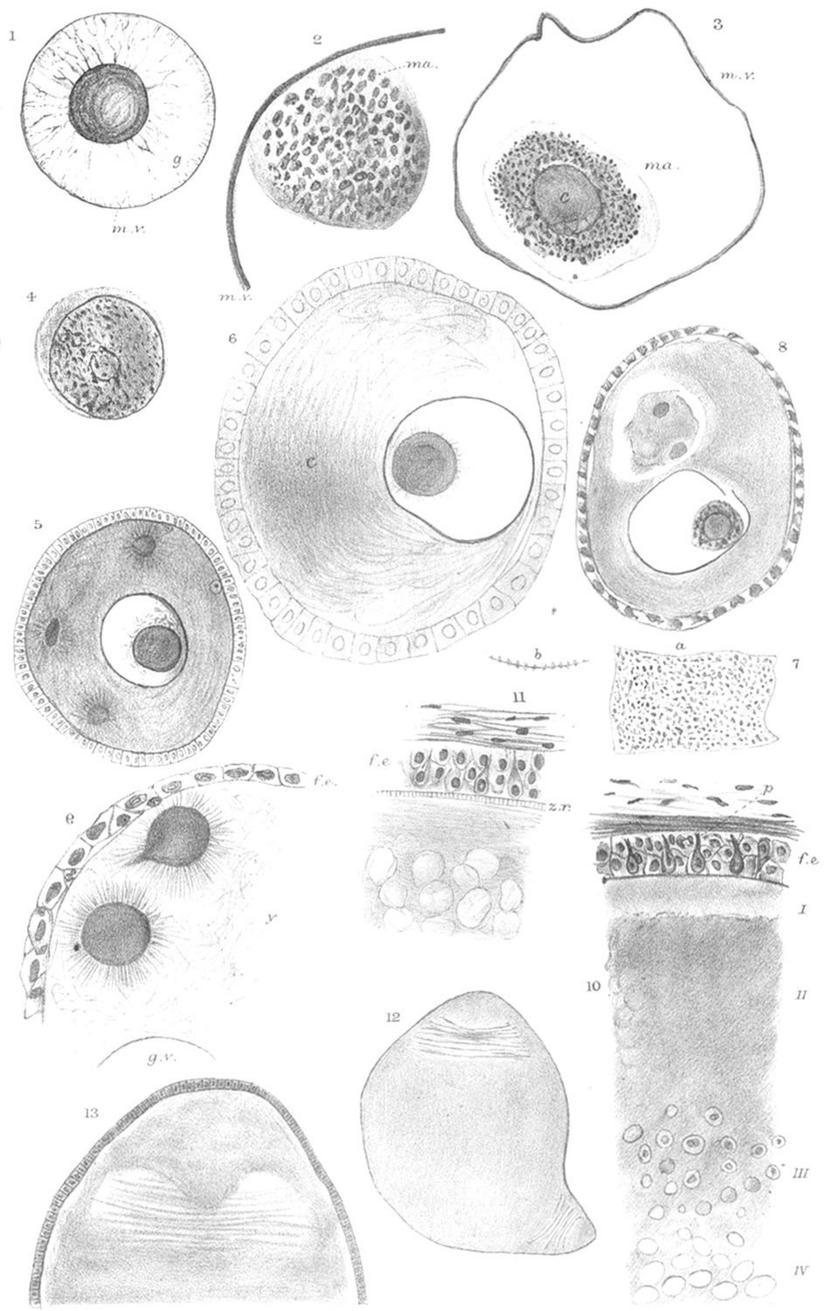


Figs. 1 to 13 are from sections of hens' ovaries, portions of which were hardened first in picric acid, and subsequently in alcohol. The sections were made after imbedding by the method of soaking with cacao-butter ("Course of Practical Histology," p. 92), and were stained either with logwood or with magenta.

Fig. 1. Germinal vesicle, germinal spot, and intra-vesicular network of a small ovarian ovum (·305 millim. in diameter). Magnified 250 diameters.

m. v. Membrane of germinal vesicle.

g. Break in the intra-vesicular network.

Fig. 2. Germinal spot of small ovum showing matrix and imbedded granules composing the macula.

m. v. Membrane of germinal vesicle to which the macula is adherent.

ma. Homogeneous matrix of germinal spot in which darkly stained granules are imbedded.

Diameter of ovum ·488 millim. Magnified 250 diameters.

Fig. 3. Germinal vesicle and germinal spot of a very small ovum.

m. v., ma., as before.

c. Central clear part of macula. (Diameter of ovum ·122 millim.; of germinal vesicle ·054 millim.) Magnified about 600 diameters.

Fig. 4. *Macula germinativa* of a very small ovum. (Diameter of ovum ·122 millim.; of macula ·049 millim.) Magnified 400 diameters.

Fig. 5. Small Graafian follicle with ovum, showing two or three nucleus-like condensations of the vitellus. (Diameter of ovum ·3 millim.) Magnified 120 diameters.

Fig. 6. Smaller ovum (·264 millim.) than that represented in fig. 5, but more highly magnified, showing a condensation, *c*, of the vitelline network.

Fig. 7. Small piece of the wall of the germinal vesicle of a moderately sized ovum (1·07 millim., germinal vesicle ·195 millim).

(*a.*) On the flat.

(*b.*) Seen in optical section.

The membrane is seen to have a granular appearance.

Fig. 8. Very small ovarian ovum (diameter ·11 millim.) with follicular epithelium, germinal vesicle, and germinal spot; and with a peculiar insulated body in the vitellus, which is apparently the remains of a second germinal vesicle and spot in process of disappearance.

Fig. 9. Part of a small ovum (·3 millim.) with two pseudo-nuclei in vitellus.

f. e. Follicular epithelium.

y. Yolk substance forming a network.

g. v. Part of the germinal vesicle.

Fig. 10. Superficial layers of a comparatively large ovum (1·586 millim.)

f. e. Follicular epithelium.

p. Pyriform cell.

I. Clear superficial layer of vitellus; II. Finely granular layer—its granules are largest near the first layer; III. White yolk; IV. Superficial part of yellow yolk. Drawn under a magnifying power of about 500 diameters.

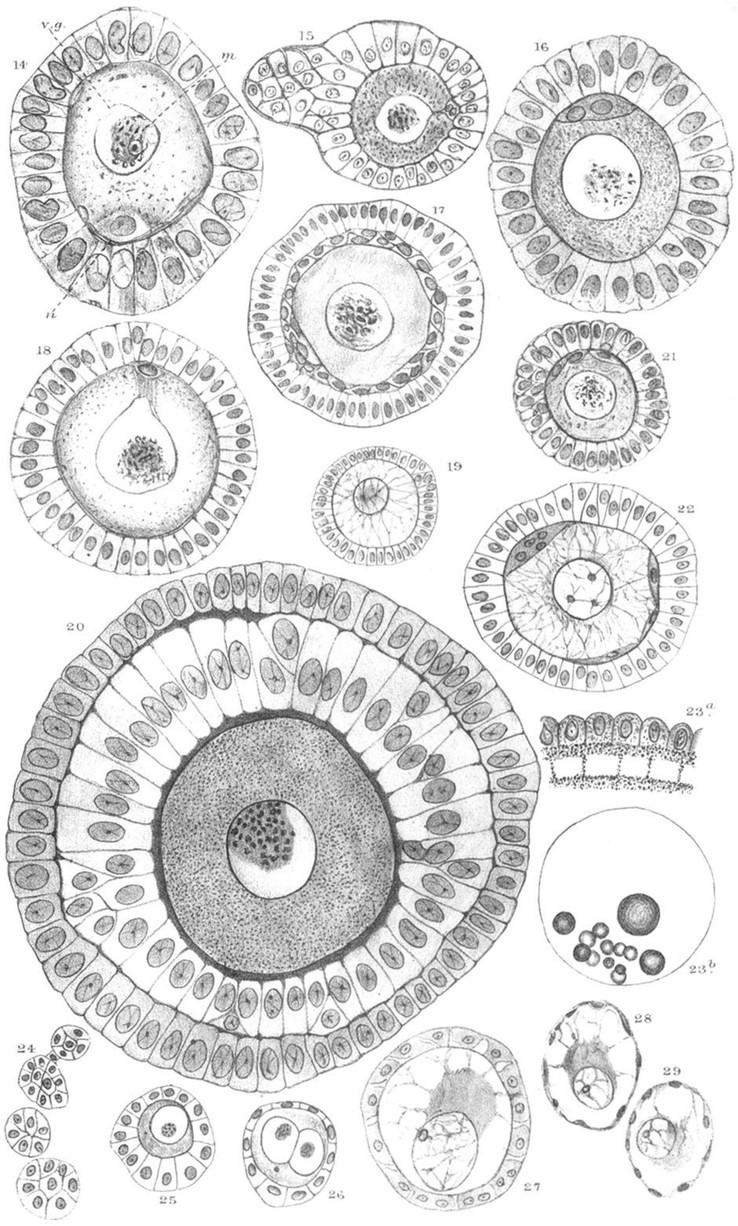
Fig. 11. Superficial part of a still larger ovum (2·44 millim.).

f. e. Follicular epithelium.

z. r. Zona radiata.

Fig. 12. Thick section of a comparatively large ovum, showing the peculiar systems of fibres described in the text. Slightly magnified (about 10 diameters).

Fig. 13. The upper part of the previous figure. Magnified 40 diameters.



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v. g. Germinal vesicle, the wall of which seems to be deficient at this part.

m. Macula, composed of a mass of granules, imbedded in a homogeneous matrix.

n. Nucleus in the periphery of the vitellus. 570 diameters.

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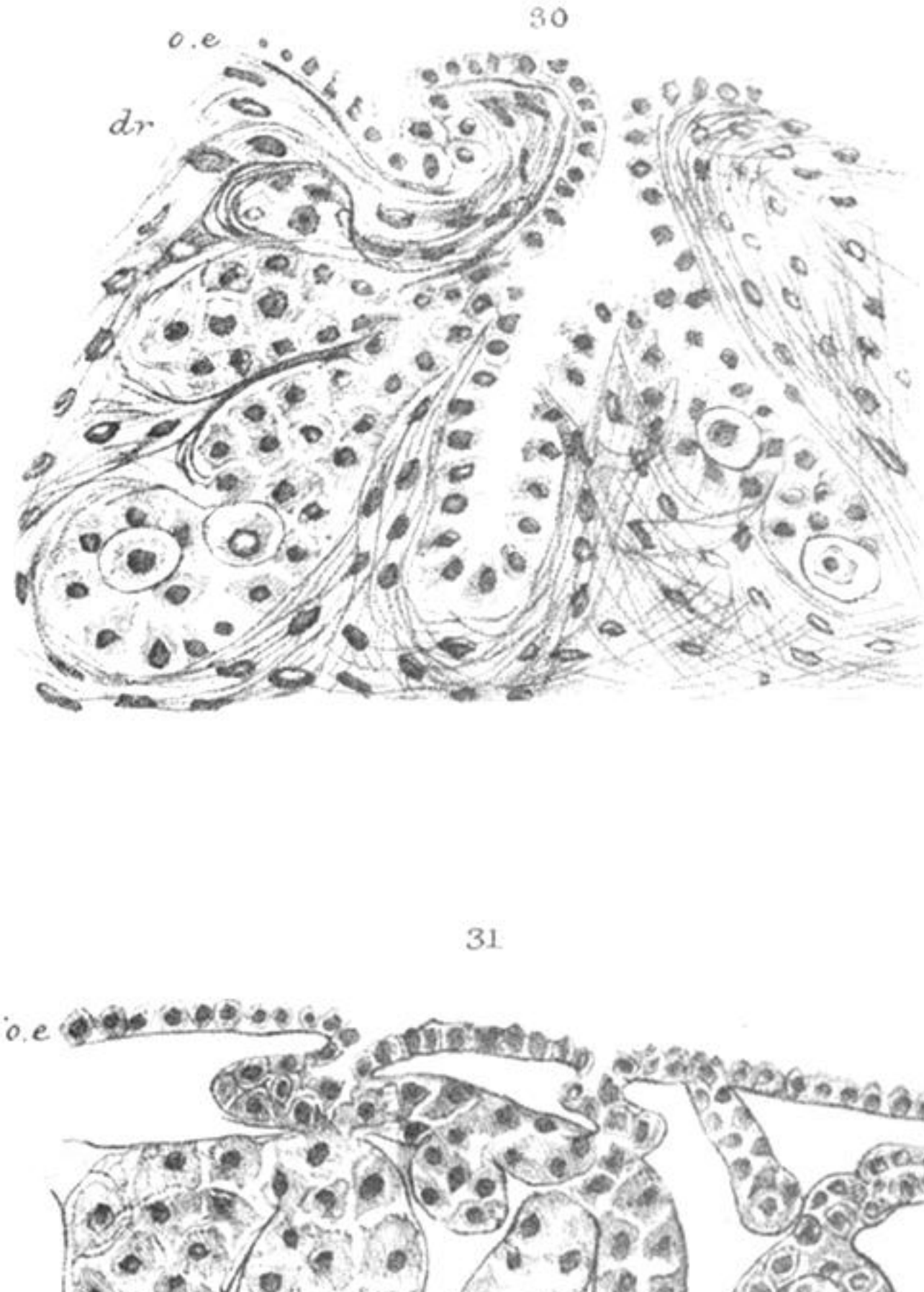

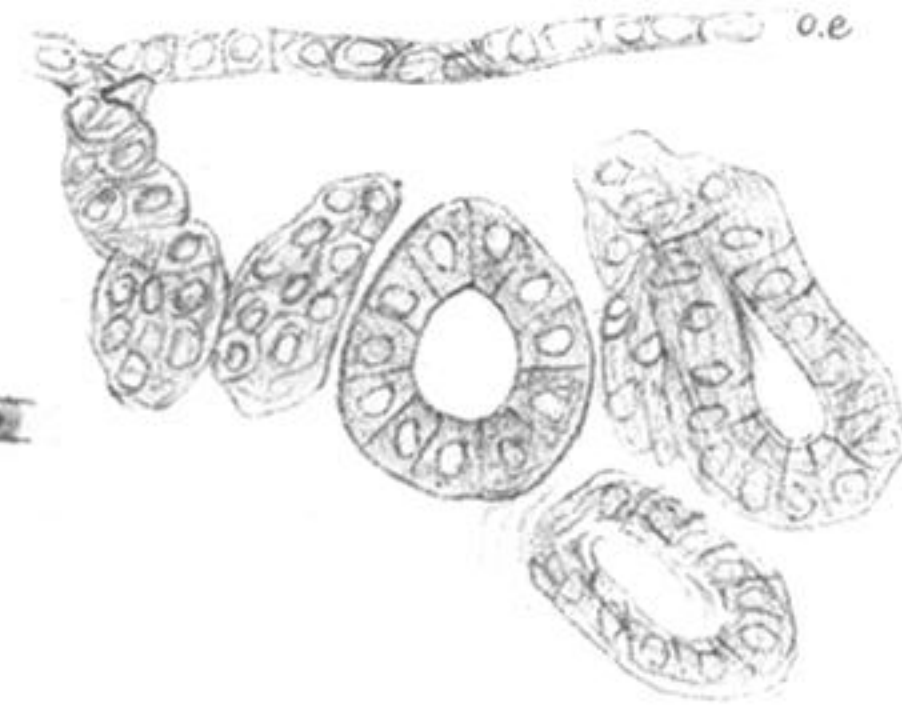

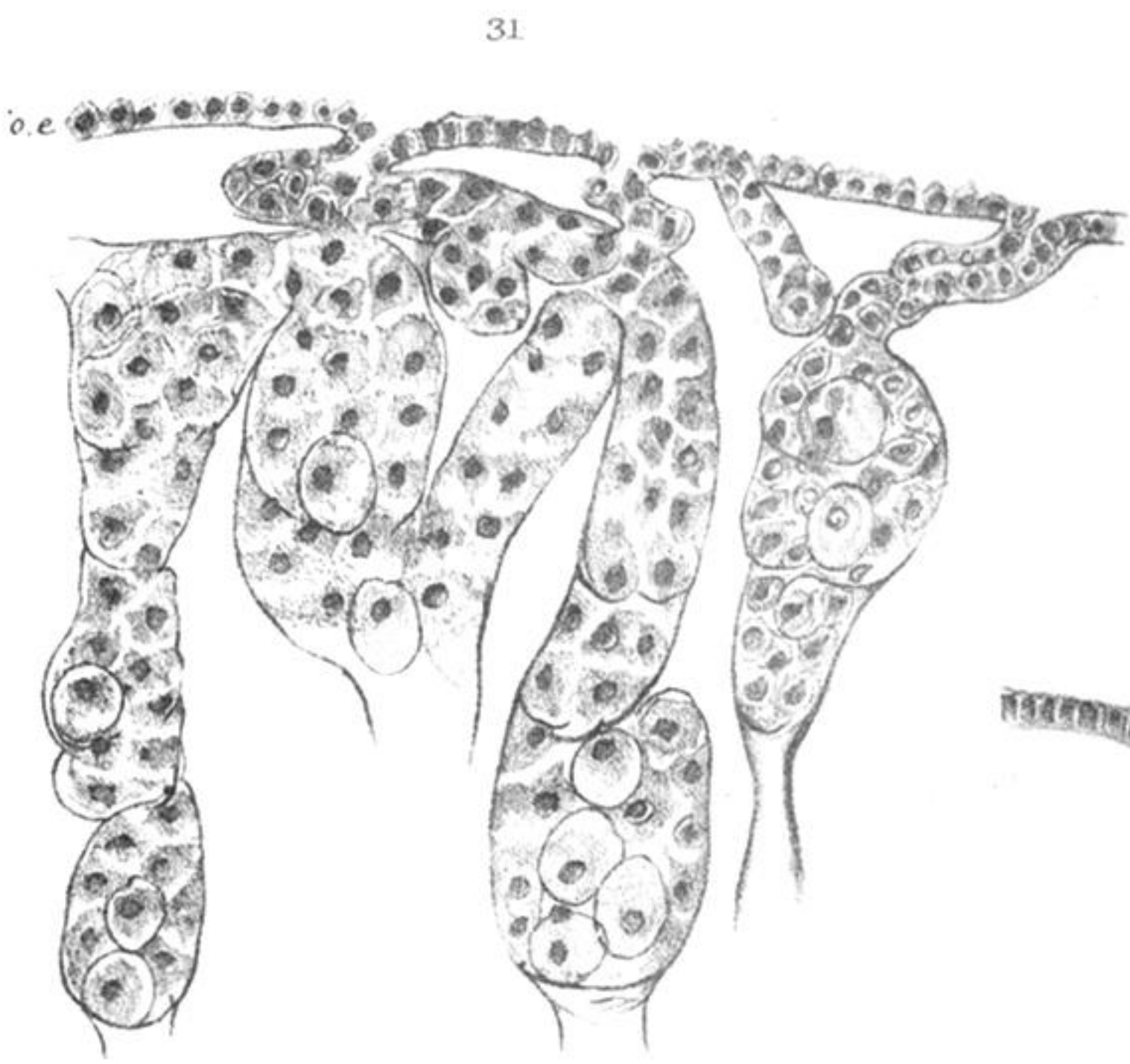

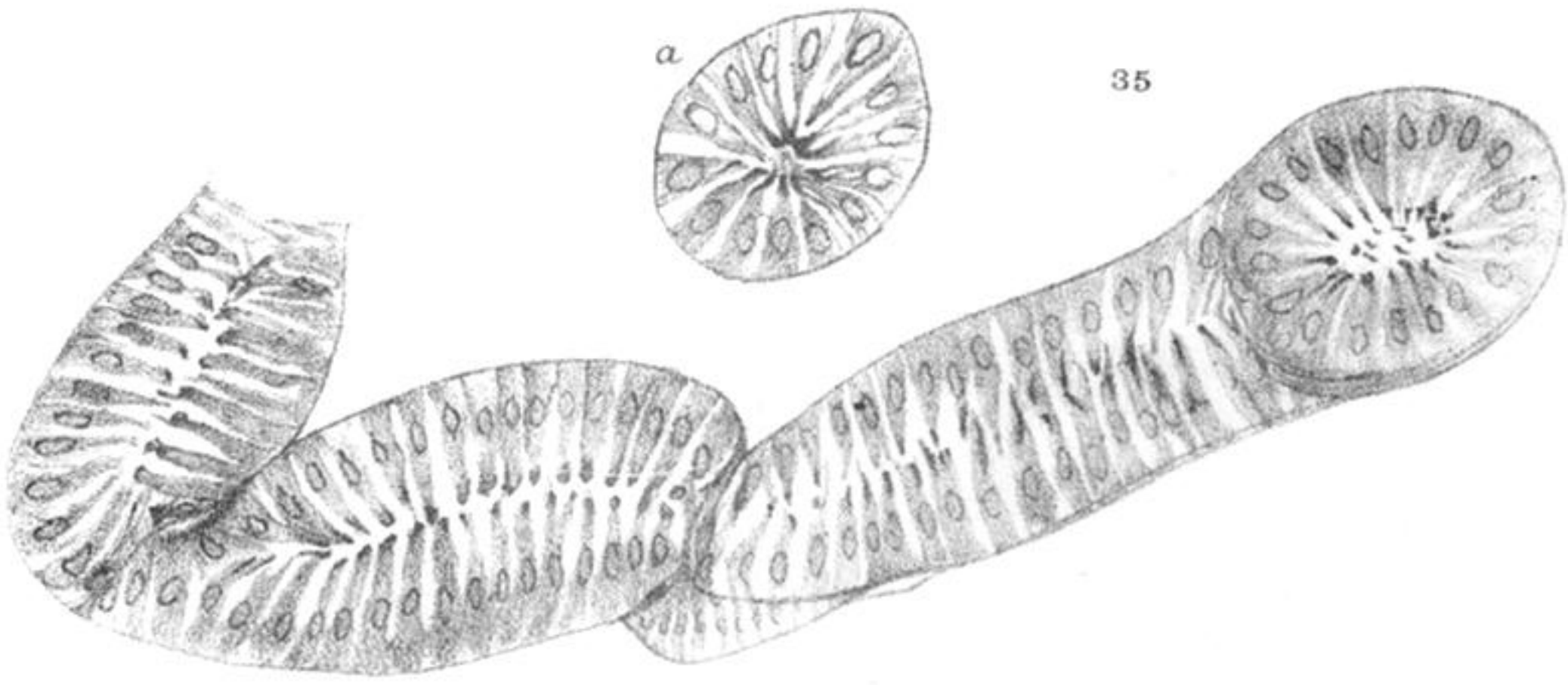

Fig. 30. Section of superficial part of ovary of a bitch pup, eighteen days old, showing gland-like connexion of egg-tubes with the surface of the ovary.

str. Ovarial stroma.

Fig. 31. From another section of the same ovary, showing more oblique connexion of egg-tubes with the surface.

o. e. Ovarial epithelium. Stroma not represented.

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a. Either the same or a similar tube, cut across.